AMENDMENTS TO THE CLAIMS

This listing of claims replaces all previously submitted listing of claims:

1. (Currently amended) A method for producing a ~~human-like~~ recombinant glycoprotein in a non-human eukaryotic host cell that expresses a glycosidase activity, the method comprising the step of diminishing or depleting the activity of one or more enzymes in the host cell that transfers a sugar residue to the 1,6 arm of a lipid-linked oligosaccharide structure; wherein said method results in the production within the host cell of recombinant glycoproteins having N-glycans attached thereto comprising $GlcNAcMan_xGlcNAc_2$ core structures, wherein X is 3, 4, or 5.

2. (Currently amended) The method of claim 1, ~~further comprising the step of introducing into the host cell at least one glycosidase activity~~ wherein the at least one glycosidase activity is expressed from a nucleic acid molecule introduced into the host cell.

3. (Currently amended) The method of claim 2, wherein the at least one glycosidase activity is a mannosidase activity.

4. (Previously presented) The method of claim 1, further comprising producing an N-glycan.

5. (Canceled)

6. (Currently amended) The method of claim ~~5~~ 1, further comprising the step of expressing within the host cell one or more enzyme activities, selected from glycosidase and glycosyltransferase activities, to produce a $GlcNAc_2Man_3GlcNAc_2$ structure.

7. (Currently amended) The method of claim 6, wherein the ~~activity~~ one or more enzyme activities is selected from α-1,2 mannosidase, α-1,3 mannosidase and GnTII activities.

8. (Previously presented) The method of claim 1, wherein at least one diminished or depleted enzyme is selected from the group consisting of an enzyme having dolichyl-P-Man:$Man_5GlcNAc_2$-PP-dolichyl alpha-1,3 mannosyltransferase activity; an enzyme having dolichyl-P-Man:$Man_6GlcNAc_2$-PP-dolichyl alpha-1,2 mannosyltransferase activity and an enzyme having dolichyl-P-Man:$Man_7GlcNAc_2$-PP-dolichyl alpha-1,6 mannosyltransferase activity.

9. (Previously presented) The method of claim 1, wherein the diminished or depleted enzyme has dolichyl-P-Man:$Man_5GlcNAc_2$-PP-dolichyl alpha-1,3 mannosyltransferase activity.

10. (Previously presented) The method of claim 1, wherein the enzyme is diminished or depleted by mutation of a host cell gene encoding the enzymatic activity.

11. (Previously presented) The method of claim 10, wherein the mutation is a partial or total deletion of a host cell gene encoding the enzymatic activity.

12. (Currently amended) The method of claim 1, wherein the ~~glycoprotein comprises~~ attached *N*-glycans ~~having~~ have seven or fewer mannose residues.

13. (Canceled)

14. (Previously presented) The method of claim 1, wherein the glycoprotein comprises one or more sugars selected from the group consisting of galactose, GlcNAc, sialic acid, and fucose.

15. (Previously presented) The method of claim 1, wherein the glycoprotein comprises at least one oligosaccharide branch comprising the structure NeuNAc-Gal-GlcNAc-Man.

16. (Previously presented) The method of claim 1, wherein the host is a lower eukaryotic cell.

17. (Previously presented) The method of claim 1, wherein the host cell is selected from the group consisting of *Pichia pastoris, Pichia finlandica, Pichia trehalophila, Pichia koclamae, Pichia membranaefaciens, Pichia opuntiae, Pichia thermotolerans, Pichia salictaria, Pichia guercuum, Pichia pijperi, Pichia stiptis, Pichia methanolica, Pichia sp., Saccharomyces cerevisiae, Saccharomyces sp., Hansenula polymorpha, Kluyveromyces sp., Candida albicans, Aspergillus nidulans, Aspergillus niger, Aspergillus oryzae, Trichoderma reesei, Chrysosporium lucknowense, Fusarium sp., Fusarium gramineum, Fusarium venenatum* and *Neurospora crassa.*

18. (Previously presented) The method of claim 1, wherein the host cell is further deficient in expression of initiating α-1,6 mannosyltransferase activity.

19. (Previously presented) The method of claim 18, wherein the host cell is an OCH1 mutant of *P. pastoris.*

20. (Previously presented) The method of claim 1, wherein the host cell expresses GnTI and UDP-GlcNAc transporter activities.

21. (Previously presented) The method of claim 1, wherein the host cell expresses a UDP- or GDP-specific diphosphatase activity.

22. (Previously presented) The method of claim 1, further comprising the step of isolating the glycoprotein from the host.

23. (Previously presented) The method of claim 22, further comprising the step of subjecting the isolated glycoprotein to at least one further glycosylation reaction *in vitro*, subsequent to its isolation from the host.

24. (Previously presented) The method of claim 1, further comprising the step of introducing into the host a nucleic acid molecule encoding one or more enzymes involved in the production of $GlcNAcMan_3GlcNAc_2$ or $GlcNAc_2Man_3GlcNAc_2$.

25. (Previously presented) The method of claim 24, wherein at least one of the enzymes has mannosidase activity.

26. (Previously presented) The method of claim 25, wherein the enzyme has an α-1,2-mannosidase activity and is derived from mouse, human, *Lepidoptera*, *Aspergillus nidulans*, *C. elegans*, *D. melanogaster*, or *Bacillus* sp.

27. (Previously presented) The method of claim 25, wherein the enzyme has an α-1,3-mannosidase activity.

28. (Previously presented) The method of claim 24, wherein at least one enzyme has glycosyltransferase activity.

29. (Previously presented) The method of claim 28, wherein the glycosyltransferase activity is selected from the group consisting of GnTI and GnTII.

30. (Previously presented) The method of claim 24, wherein at least one enzyme is localized by forming a fusion protein between a catalytic domain of the enzyme and a cellular targeting signal peptide.

31. (Previously presented) The method of claim 30, wherein the fusion protein is encoded by at least one genetic construct formed by the in-frame ligation of a DNA fragment encoding a cellular targeting signal peptide with a DNA fragment encoding a glycosylation enzyme or catalytically active fragment thereof.

32. (Previously presented) The method of claim 31, wherein the encoded targeting signal peptide is derived from a member of the group consisting of mannosyltransferases, diphosphotases, proteases, GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI, GalT, FT, and ST.

33. (Currently amended) The method of claim 31, wherein the catalytic domain encodes a glycosidase or glycosyltransferase that is derived from a member of the group consisting of GnT I, GnT II, GnT III, GnT IV, GnT V, GnT VI, GalT, Fucosyltransferase and ST~~, and wherein~~

~~the catalytic domain has a pH optimum within 1.4 pH units of the average pH optimum of other representative enzymes in the organelle in which the enzyme is localized, or has optimal activity at a pH between 5.1 and 8.0~~.

34. (Previously presented) The method of claim 31, wherein the nucleic acid molecule encodes one or more enzymes selected from the group consisting of UDP-GlcNAc transferase, UDP-galactosyltransferase, GDP-fucosyltransferase, CMP-sialyltransferase, UDP-GlcNAc transporter, UDP-galactose transporter, GDP-fucose transporter, CMP-sialic acid transporter, and nucleotide diphosphatases.

35. (Previously presented) The method of claim 31, wherein the host expresses GnTI and UDP-GlcNAc transporter activities.

36. (Previously presented) The method of claim 31, wherein the host expresses a UDP- or GDP-specific diphosphatase activity.

37. (Currently amended) The method of claim 1, further comprising the step of introducing into a host that is deficient in dolichyl-P-Man:Man5GlcNAc2-PP-dolichyl alpha-1,3 mannosyltransferase activity a nucleic acid molecule encoding one or more enzymes for production of ~~a GlcNAcMan$_4$GlcNAc$_2$ carbohydrate structure~~ the N-glycans comprising GlcNAcMan$_x$GlcNAc$_2$ core structures.

38. (Currently amended) The method of claim 1, further comprising the step of introducing into a host that is deficient in dolichyl-P-Man:Man6GlcNAc2-PP-dolichyl alpha-1,2 mannosyltransferase or dolichyl-P-Man:Man7GlcNAc2-PP-dolichyl alpha-1,6 mannosyltransferase activity a nucleic acid molecule encoding one or more enzymes for production of ~~a GlcNAcMan$_4$GlcNAc$_2$ carbohydrate structure~~ the N-glycans comprising GlcNAcMan$_x$GlcNAc$_2$ core structures.

39. (Previously presented) The method of claim 37 or 38, wherein the nucleic acid molecule encodes at least one enzyme selected from the group consisting of an α-1,2 mannosidase, UDP GlcNAc transporter and GnT1.

40. (Currently amended) The method of claim 39, further comprising the step of introducing into the deficient host cell a nucleic acid molecule encoding an α-1,3 or an α-1,2/α-1,3 mannosidase activity for the conversion of the $GlcNAc_1Man_4GlcNAc_2$ structure to a ~~$GlcNAc_1Man_3GlcNAc_2$~~ $GlcNAcMan_3GlcNAc_2$ structure.

41. (Previously presented) The method of claim 1, further comprising the step of introducing into the host a nucleic acid molecule encoding one or more enzymes for production of a $GlcNAc_2Man_3GlcNAc_2$ carbohydrate structure.

42. (Previously presented) The method of claim 41, wherein at least one enzyme is GnTII.

43. (Currently amended) The method of claim 1, further comprising the step of introducing into the host cell at least one nucleic acid molecule encoding at least one mammalian glycosylation enzyme selected from the group consisting of a glycosyltransferase, fucosyltransferase, ~~glactosyltransferase~~ galactosyltransferase, N-acetylgalactosaminyltransferase, N-acetylglycosaminyltransferase and sulfotransferase.

44. (Previously presented) The method of claim 1, comprising the step of transforming host cells with a DNA library to produce a genetically mixed cell population expressing at least one glycosylation enzyme derived from the library, wherein the library comprises at least two different genetic constructs, at least one of which comprises a DNA fragment encoding a cellular targeting signal peptide ligated in-frame with a DNA fragment encoding a glycosylation enzyme or catalytically active fragment thereof.

45. (Previously presented) A host cell produced by the method of claim 1 or 44.

46. (Previously presented) A human-like glycoprotein produced by the method of claim 1 or 44.

47. (Withdrawn) A nucleic acid molecule comprising or consisting of at least forty-five consecutive nucleotide residues of Fig. 6 (*P. pastoris ALG 3* gene).

48. (Withdrawn) A vector comprising a nucleic acid molecule of claim 47.

49. (Withdrawn) A host cell comprising a nucleic acid molecule of claim 47.

50. (Withdrawn) A *P.pastoris* cell in which the sequences of Fig. 6 (*P. pastoris ALG 3* gene), are mutated whereby the glycosylation pattern of the cell is altered.

51. (Withdrawn) A method to enhance the degree of glucosylation of lipid-linked oligosaccharides comprising the step of increasing alpha-1,3 glucosyltransferase activity in a host cell.

52. (Withdrawn) A method to enhance the degree of glucosylation of lipid-linked oligosaccharides comprising decreasing the substrate specificity of oligosaccharyl transferase activity in a host cell.

53. (Withdrawn) A method for producing in a non-mammalian host cell an immunoglobulin polypeptide having an N-glycan comprising a bisecting GlcNAc, the method comprising the step of expressing in the host cell a GnTIII activity.

54. (Withdrawn) A non-mammalian host cell that produces an immunoglobulin having an N-glycan comprising a bisecting GlcNAc.

55. (Withdrawn) An immunoglobulin produced by the host cell of claim 54.

56. (Withdrawn) A method for producing in a non-human host cell a polypeptide having an N-glycan comprising a bisecting GlcNAc, the method comprising the step of expressing in the host cell a GnTIII activity.

57. (Withdrawn) A non-human host cell that produces a polypeptide having an N-glycan comprising a bisecting GlcNAc.

58. (Withdrawn) A polypeptide produced by the host cell of claim 57.

59. (Previously presented) A method for producing a human-like glycoprotein in a non-human eukaryotic host cell comprising the step of diminishing or depleting from the host cell an *alg* gene activity and introducing into the host cell at least one glycosidase activity.

60. (Withdrawn) A method for producing a human-like glycoprotein having an N-glycan comprising at least two GlcNAcs attached to a trimannose core.

61. (New) The method of claim 1, wherein the host cell further expresses a GnTIII activity.

62. (New) The method of claim 1, wherein the recombinant glycoprotein is an immunoglobulin.

63. (New) The method of claim 61 or 62, wherein the recombinant glycoprotein is an immunoglobulin comprising a bisecting GlcNAc.

64. (New) The method of claim 37 or 38, wherein the $GlcNAcMan_xGlcNAc_2$ core structures comprise predominantly $GlcNAcMan_4GlcNAc_2$.

65. (New) The method of claim 37 or 38, wherein the $GlcNAcMan_xGlcNAc_2$ core structures comprise predominantly $GlcNAcMan_3GlcNAc_2$.